

Assessment of 1,3-Butadiene Exposure in Polymer Production Workers Using *HPRT* Mutations in Lymphocytes as a Biomarker

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1,3-Butadiene (BD), which is used to make styrene-butadiene rubber, is a potent carcinogen in mice and a probable carcinogen, associated with leukemia, in humans. We have previously used *HPRT* mutation as a biomarker to evaluate exposures to BD in a monomer production plant. We now report on a study of 49 workers in a styrene-butadiene rubber plant in which we used the concentration of the BD metabolite 1,2-dihydroxy-4-(*N*-acetylcysteinyl-*S*)-butane (M1) in urine as a biomarker of exposure and the frequency of *HPRT* variant (mutant) lymphocytes (Vf) as a biomarker of effect. Workers were assigned to high- and low-exposure groups based on historical information about work areas and jobs. Personal exposure to BD for one work shift was measured using a passive badge dosimeter. Each participant provided a urine specimen and blood sample at the end of the work shift and completed a questionnaire providing information on lifestyle, health, and work activities. The average BD exposures in the high- and low-exposure groups were significantly different, even after excluding two extreme values, (high 1.48 ppm; low 0.15 ppm, $p < 0.002$). This study was done in 1994 and 1995 before the establishment, in 1996, of the new permissible exposure limit of 1 ppm. Both the mean M1 and the *HPRT*Vf were more than three times greater in the high-exposure group than in the low-exposure group ($p < 0.0005$). The three end points correlated with each other, with sample correlation coefficients between 0.4 and 0.6. The correlations among BD exposure and the biomarkers of internal exposure and genotoxicity suggest that occupational exposure to BD, in the range of 1–3 ppm, may be associated with adverse biological effects. **Key words:** biological monitoring, butadiene, *HPRT* mutations, occupational health. *Environ Health Perspect* 110:1249–1255 (2002). [Online 28 November 2001] <http://ehpnet1.niehs.nih.gov/docs/2002/110p1249-1255ammenheuser/abstract.html>

Butadiene (BD), a flammable gas with a pungent odor, is used in the manufacture of styrene-butadiene rubber and other polymers. BD is a four-carbon compound with two double bonds that can be oxidized to epoxides, which can then rearrange to form reactive metabolites. In 1995 BD ranked 36th in U.S. production volume at 3.68 billion pounds (1).

Butadiene is carcinogenic in rodents. Female mice developed lung tumors after chronic exposure of only 6.25 ppm. At higher dose levels the mice developed tumors at multiple sites, including the hematopoietic system, lungs, forestomach, heart, mammary glands, and ovaries (2). Rats appear to be less sensitive but developed tumors in several organs including the pancreas, testes, uterus, and the mammary, zymbal, and thyroid glands at chronic exposure levels of 1,000 ppm (3).

In human epidemiologic studies there have been a number of reports of increased mortality rates in BD-exposed workers due to malignancies of the hematopoietic system (4). In these studies, however, there have been inconsistencies in the types of malignant tumors and in the relationship of tumor incidence to BD exposure level, duration of exposure, and time period of exposure (World War II vs. the postwar period) (5,6).

Butadiene has been shown to be genotoxic in both *in vitro* and *in vivo* laboratory tests. BD was mutagenic in *Salmonella typhimurium* strain TA 1530 with metabolic activation (7), and BD gas was weakly mutagenic in the mouse lymphoma assay in the presence of rat liver S9 (8). In laboratory animals, BD can induce chromosomal damage and somatic cell mutations. The most sensitive cytogenetic end point in mice appears to be sister chromatid exchanges. These were induced in bone marrow by a 10-day exposure to 6.25 ppm BD (9). In the same study, bone marrow micronuclei were induced at 62.5 ppm and chromosome aberrations at 625 ppm (9). At BD doses as low as 50 ppm for 5 days, micronuclei were induced in both bone marrow and peripheral blood erythrocytes (10). Exposure of mice to BD doses as low as 20 ppm for 4 weeks induced gene mutations at the *HPRT* locus in spleen lymphocytes (11,12) and in thymic lymphocytes (12). In transgenic mice, exposures to 62.5 ppm of BD for 4 weeks resulted in increased frequencies of mutations at the *lacI* locus in bone marrow cells (13). Rats appear to be much less sensitive to BD-induced chromosomal damage (14) and mutation (12) when compared to mice. This is consistent with the observed carcinogenic effects in the two species.

The carcinogenic and mutagenic effects of BD are thought to be due to the formation of the epoxide metabolites butadiene monoepoxide (EB), butadiene diepoxide (DEB), and butadiene diolepoxide (EBD). All three epoxide metabolites are mutagenic both *in vitro* and *in vivo*. Of the three, DEB is the most potent mutagen; it is 4–12 times more potent than EB in the Ames/*Salmonella* assay (15) and 100 times more potent in human TK6 lymphoblasts (16). EBD was of intermediate potency in these *in vitro* assays (15,16). *In vivo*, the three epoxides induced increased frequencies of bone marrow micronuclei in the following order of potency: DEB > EB > EBD (15). The mutagenic potency of BD, EB, and DEB at the *HPRT* locus was determined in mice and rats by Meng et al. (17). EB was mutagenic in mice but not in rats, whereas DEB was more potent in rats than in mice.

The differences in carcinogenic responses between rats and mice and weaknesses in some of the human epidemiologic studies have resulted in controversies regarding the probable carcinogenic risks of BD to humans (6,18). It is difficult to know which animal model might best be used to estimate human risk (if either), and it is unlikely that the epidemiologic record can be improved in the near future. Industry responded in the late 1980s to concerns about the carcinogenicity of BD by initiating steps to reduce exposures. The Occupational Safety and Health Administration (OSHA) promulgated a new permissible exposure limit in 1996, reducing the 8-hr time-weighted average exposure limit from 1,000 ppm BD to 1 ppm (19). New epidemiologic studies, designed to detect carcinogenic effects at these low concentrations,

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would require very large cohorts to achieve adequate statistical power. Also, disease outcomes following extended chronic exposure, based on workers not exposed to earlier higher concentrations of BD, will not be available for several decades.

A more appropriate approach for current investigations of the effects of BD exposure would be to use biological markers as outcome measures in human population studies. Because BD and its metabolites are genotoxic in animal studies, genotoxicity tests can be useful as effect biomarkers in studies of populations of exposed workers. Also, by using a biomarker of internal exposure, such as the level of a urinary metabolite of BD, associations between exposure and the genotoxic effects of exposure can be identified. Although somatic cell genotoxicity is not an adverse health outcome in itself, genotoxic effects are mechanistically linked to neoplasia. Because of this linkage, evidence that exposure to a suspected carcinogen is associated with genotoxicity suggests that a preventable risk might exist. In the early 1990s we conducted a preliminary study of a small cohort of workers in a BD monomer plant. We found that workers with exposure of about 1–3 ppm BD had a 3-fold elevation in the frequency of variant (mutant) lymphocytes in the autoradiographic *HPRT* mutant lymphocyte assay compared to less-exposed workers and nonexposed, outside controls (20). This increase correlated with an elevation in the concentration of a conjugated metabolite derived from EB [1,2-dihydroxy-4-(*N*-acetylcysteiny1-*S*)-butane], called “M1.” No increase was observed in chromosome aberrations using conventional cytogenetic assays, although a small increase in chromosomal damage was detected in cells from the higher exposed group compared to the low- or nonexposed groups after an *in vitro* challenge with X rays (21). A follow-up study at this same monomer plant a few months later confirmed the observation of an increased *HPRT* variant frequency (Vf) (22).

We have now conducted a study of a larger group of workers in a styrene-butadiene rubber manufacturing facility in southeast Texas. Our objective was to determine whether the earlier observations could be confirmed in a different occupational setting. In this study, conducted in 1994–1995, the exposure of workers to BD was measured using personal organic vapor monitors. The urine M1 metabolite of BD, and the frequency of *HPRT* mutant lymphocytes were measured as biomarkers of internal exposure and effect, respectively. We report here that, in workers with higher exposures to BD, we confirm our earlier observation of an increased urine M1 concentration and an elevation in *HPRT* Vf.

Materials and Methods

Chemicals and media. Reagents and media for cell culture were obtained from the following suppliers: 6-thioguanine (TG), citric acid, and dimethyl sulfoxide (DMSO) were obtained from Sigma (St. Louis, MO); phytohemagglutinin (PHA), reagent grade, from Murex (HA 15, 45 mg/5 mL bottle; Dartford, England), RPMI 1640 medium, penicillin-streptomycin, and L-glutamine from GIBCO-BRL (Gaithersburg, MD); HL-1 medium supplement from Bio-Whittaker (Walkersville, MD); and fetal bovine serum (FBS) from HyClone Laboratories (Logan, UT). NTB-2 autoradiographic emulsion and D-19 developer were obtained from Kodak (Rochester, NY) and [³H]-thymidine (³H-TdR) was obtained from ICN (Costa Mesa, CA).

Study design. This study was designed to evaluate the possible genotoxic effects of working in a styrene-butadiene rubber plant in southeast Texas. Workers were recruited with a letter that explained the purpose and design of the study and asked for their participation. Those who agreed to participate completed a short form attached to the letter to enroll in the study. Each volunteer also signed a consent form approved by the human subject Institutional Review Board of the University of Texas Medical Branch. Participants then completed a questionnaire which provided information on the following subjects: personal description (age, ethnicity, sex); history of tobacco use and customary use of alcohol and caffeine-containing beverages; health status and medication use; and work-related information including job title, work locations, and occupational history. Each participant was asked to wear a 3M 3520 organic vapor monitor (3M, St. Paul, MN), which served as a passive dosimeter, for one work shift (8 hr) to measure exposure to butadiene and styrene. At the end of the shift, a urine specimen and a blood sample of approximately 70 mL was collected. Before the collection of personal exposure data and biological samples, we divided the workers into two groups based on historical butadiene exposure levels in different work areas. A pilot exposure assessment was consistent with this assignment. Workers in the reactor, recovery, tank farm, and laboratory areas were assigned to the high-exposure group. Workers in the blending, coagulation, baling, shipping, control room, and utility areas were assigned to the low-exposure group. The primary end points analyzed were the time-weighted average exposure to butadiene in breathing zone air, the concentration of the M1 metabolite in urine, and the *HPRT* Vf in lymphocytes. We confirmed smoking status by measuring cotinine in blood plasma.

Exposure assessment. Organic vapor monitors (OVM; 3M 3520) with two charcoal layers were used as passive badge dosimeters. The sealed containers were opened immediately before giving the monitor to a participant, who wore it attached to clothing near the face for the entire work shift. Workers carried a two-page diary form during the day to record their work location and activities for each hour of the shift. For each hour of the shift, the workers were asked to characterize their chemical exposure as about typical for their jobs, higher, or lower than typical. They were also asked to indicate whether they wore a respirator during that hour and to record any unusual events. At the end of the shift the workers reported back to the study site and turned in the OVM, which was immediately capped, placed in its canister, and stored in an ice chest. The OVMs were shipped to an analytical laboratory (NATELSO, Long Grove, IL) for extraction and analysis by gas chromatography. The minimum time-weighted average exposure that could be reliably calculated with this method was about 0.25 ppm for butadiene and about 1 ppm for styrene.

Urine analysis. A single urine specimen of up to 200 mL was collected in one or two polypropylene specimen containers at the end of the work shift. Specimens were immediately frozen on dry ice. They were stored in the laboratory at –20°C until shipped on dry ice to the Inhalation Toxicology Research Institute (now Lovelace Respiratory Research Institute) in Albuquerque, New Mexico. They were analyzed there for the M1 metabolite as described previously (23). Briefly, deuterated standards were added to aliquots of the samples, which were then extracted and analyzed by selective ion monitoring in a Hewlett-Packard gas chromatograph, mass spectrometer (Hewlett-Packard, Palo Alto, CA). Creatinine levels were also determined to normalize for the dilution effects of fluid consumption.

Lymphocyte and plasma isolation and cryopreservation. A blood sample from each worker was collected in five 15-mL sodium heparinized vacuum tubes (Becton-Dickinson, Franklin Lakes, NJ). Samples were returned to the laboratory within 4–12 hr of collection. Mononuclear cells and plasma were separated from the whole blood by density centrifugation on Histopaque (Sigma). The lymphocytes were washed, counted, and cryopreserved, as previously described (24). Cells were frozen at 10–15 × 10⁶ cells per 1-mL cryotube in 50% FBS, 10% DMSO, and 40% RPMI 1640. An average of 6 cryotubes per subject were prepared and stored in liquid nitrogen until ready for use.

A 2-mL aliquot of plasma from each blood sample was frozen at –20°C in glass

vials and later shipped on dry ice to the laboratory of Helen Van Vunakis (Brandeis University, Waltham, MA). The plasma was analyzed for cotinine concentration by radioimmunoassay (25). Cotinine concentrations > 20 ng/mL of plasma were considered indicative of active tobacco use. The remaining plasma from the blood was frozen for later use as an autologous growth supplement in the *HPRT* assay.

The *HPRT* mutant lymphocyte assay. We determined the frequencies of *HPRT* variant (mutant) lymphocytes using the short-term autoradiographic assay. Detailed methods have been described previously (26,27). Assays were performed with coded samples so that the exposure status of the subjects was unknown. Three or four cryotubes (about $30\text{--}45 \times 10^6$ cells) were thawed, washed, counted, and resuspended in growth medium (RPMI 1640 with antibiotics, 2% reagent grade PHA, 20% HL-1, 25% autologous plasma). Five to 6 million lymphocytes were aliquoted into each of 5–8 vented flasks (Falcon No. 3108, Lincoln Park, NJ) and 6-thioguanine was added. The final TG concentration was 2×10^{-4} M and the final cell density was 1×10^6 /mL. One labeling index (LI) flask was prepared without TG. After a 24-hr incubation at 37°C, all cultures were labeled with 25 μ Ci of [3 H]-TdR, incubated for an additional 18 hr, and harvested by adding 9 mL of chilled 0.1 M citric acid to each flask. The free nuclei from the TG-containing flasks were washed with methanol-acetic acid fixative and resuspended in 1 or 2 tubes in 0.25 mL fixative. The nuclei from the LI flask were harvested separately using the same procedure. A 20- μ L aliquot from each tube from the TG-containing cultures was counted with a particle counter (Coulter, Hialeah, FL), and all of the remaining nuclei in the tube were placed on an 18 \times 18 mm coverslip affixed to the end of a microscope slide. The nuclei from the LI flask were counted and an aliquot of about 0.15×10^6 cells was spread evenly onto a separate 18 \times 18 mm coverslipped slide.

Slides were stained with aceto-orcein, dipped in NTB-2 emulsion, stored for 2–3 days in light-tight boxes at 4°C, and developed with D-19. Coded slides were read with a Nikon Labophot microscope (Nikon,

Tokyo, Japan), and a count was made of all labeled cells from the TG-containing cultures. For the LI slide, a random differential count was made of 3,000 labeled and unlabeled cells to provide an estimate of the proportion of normal (nonmutant) lymphocytes from each subject that were able to grow in culture. The Vf is calculated by taking the total of the labeled cells identified on the slides derived from the TG-containing cultures and dividing this number by the LI multiplied by the number of nuclei initially added to the TG slides. The denominator for this calculation is referred to as the number of evaluable cells.

Statistical analysis. The parameters obtained for analysis were time-weighted average exposure to butadiene in parts per million, concentration of the M1 metabolite in urine in nanograms per milligram creatinine, and the *HPRT* Vf in variant cells per 10^6 evaluable lymphocytes. For group comparisons, results were analyzed by exposure group and smoking status. Differences between groups were analyzed using the unpooled variance version of the independent two-sample Student's *t*-test from the Minitab statistical package (Minitab Inc., State College, PA). We also used the nonparametric Wilcoxon rank test (S-Plus, Insightful Corporation, Seattle, WA) for comparison. The relationships between parameters were analyzed for correlations by means of the Fisher *z*-test for significance and associated confidence intervals as implemented in the S-Plus statistical program

Results

Study population. A total of 63 workers (31 in high-exposure areas and 32 in low-exposure areas) initially enrolled in the study and participated in blood sample collection. Of these, seven high-exposure and six low-exposure subjects were eliminated because their lymphocyte cultures had low LIs (< 5%) and/or low cell recovery after freezing. This resulted in insufficient numbers of evaluable cells (< 0.4×10^6) available to perform an adequate assay for detecting frequencies of *HPRT* mutant cells. *HPRT* mutant frequencies were obtained from 24 high-exposure and 25 low-exposure subjects (one low-exposure nonsmoker had an adequate LI but no labeled cells on slides from the TG-containing

cultures). Badge dosimeter measurements of BD exposure were obtained from 48 workers: 24 in high-exposure and 24 in low-exposure areas. Urine analyses for M1 were obtained for 47 participants (24 high and 23 low).

The demographic data are presented in Table 1. All of the evaluated workers were either white, non-Hispanic (75.5%), or African American (24.5%). The workers in the high-exposure areas were about 5 years older and had worked in the plant about 7 years longer than the workers in the low-exposure areas. In both groups about 80% of the workers were nonsmokers. Five individuals in the high-exposure group and three in the low-exposure group were tobacco chewers (resulting in elevations of plasma cotinine levels). All other subjects classed as nonsmokers had plasma cotinine levels < 20 ng/mL.

Results by exposure groups. The results for BD exposure levels, urine metabolites, and *HPRT* mutant lymphocytes were compared for the high- and low-exposure groups (Table 2). The minimum concentration of BD in air for which a concentration could be reported, after an 8-hr measurement, was approximately 0.25 ppm. Out of 24 determinations included in the data set for workers in the high-exposure areas, 16 produced measurable values and 8 were below the detection limit. For the 24 samples from workers in the low-exposure areas, only 2 (0.27 and 0.51 ppm) were above the detection limit, and the remainder were below. The average BD exposure levels for the different work areas are shown in Figure 1. For the purpose of calculating averages, a value of one-half of the minimum detection limit (0.125 ppm) was used for subjects whose exposures were below the detection limit. Two extreme values were omitted from Figure 1. One of these involved a laboratory worker who reported spilling BD-contaminated water on his clothing. He had a badge value that day of 20.8 ppm BD. The other subject was a maintenance worker who reported high exposure to BD while opening a line. His badge value was 23 ppm, but he wore a respirator during this operation. Neither of these unusually high BD values is likely to represent day-to-day exposure or be representative of the study population as a whole.

Styrene exposures were also determined. However, the minimum concentration that could be calculated for an 8-hr exposure was 1 ppm, and most exposures were < 1 ppm. Based on the actual mass of analyte measured from each badge, the molar ratios of BD to styrene had a mean (\pm SEM) of 27.92 ± 7.22 . Thus, BD exposures were substantially greater than styrene levels.

Table 2 presents group means for the effect and exposure biomarkers. A clear distinction was observed between the high- and

Table 1. Descriptive characteristics of the study population.

	High exposure	Low exposure
Number enrolled	31	32
Number evaluated	24	25
Mean age \pm SD (<i>n</i>)	46.7 \pm 10.0 (22)	41.6 \pm 9.9 (24)
Work longevity \pm SD (<i>n</i>)	20.9 \pm 10.7 (23)	14.0 \pm 12.2 (24)
Non-Hispanic white	19	18
African American	5	7
Smokers	5	5
Nonsmokers	19	20

low-exposure areas. The mean BD exposure value (\pm SE) for the high-exposure areas was 1.48 ± 0.37 ppm (excluding the two extreme values) with a range of 0.25–5 ppm, and for the low-exposure areas was 0.15 ± 0.02 ppm (significantly different, $p < 0.002$). The median for the high-exposure areas was 0.41 ppm, and the median for the low-exposure areas was 0.125 ppm (half of the detection limit). If the two extreme values are included, the high-exposure group mean was 3.18 ± 1.23 ppm, and the median was 0.52 ppm BD. The distribution of exposure measurements was consistent with our expectations, based on information from experienced workers in the facility, and confirms our assignment of these areas as high or low exposure.

The average concentration of the M1 metabolite in the urine of workers in the high-exposure areas was 2,046 ng/mg creatinine, compared to 585 ng/mg creatinine in the low-exposure areas. This difference was highly significant ($p < 0.0004$). The frequency of *HPRT* mutant lymphocytes (mean \pm SE) was $2.10 \pm 0.2 \times 10^{-6}$ in the low-exposure group and $6.66 \pm 1.4 \times 10^{-6}$ in the high-exposure group. This difference was also highly statistically significant ($p < 0.0002$ by *t*-test and $p < 0.0001$ by the Wilcoxon test). When stratified by smoking status, which is known to affect *HPRT*Vf (27–29), there was a highly significant difference between the 19 nonsmokers in the high-exposure areas and 20 nonsmokers in the low-exposure areas ($p < 0.0005$). Tobacco smokers were identified by both questionnaire data and plasma cotinine levels > 20 ng/mL. In this study, the self-reported data and the cotinine data were concordant. The five high-exposure smokers had a Vf of 6.1×10^{-6} , almost 2-fold higher than the five low-exposure smokers (Vf 3.3×10^{-6}), but this was not statistically significant (Table 2, Figure 2).

The quality of the cultures for *HPRT*Vf analysis was high. The average LI was 0.16, and the average number of evaluable cells was 2.57×10^6 lymphocytes. These values are comparable to other studies that we have conducted (20,22,24,27). The mean LIs were identical in the two exposure groups

(0.16), and the mean numbers of evaluable cells (LI \times total cells) were 2.78×10^6 and 2.38×10^6 in the low- and high-exposure groups, respectively.

Correlation among end points. We evaluated the correlation of BD exposure with urine M1 and *HPRT*Vf. For subjects whose exposure was below the detection limit (0.25 ppm), a value equal to one-half the detection limit (0.125 ppm) was used. Figure 3 displays the relationship between urine M1 concentration and BD exposure for those subjects who had a detectable BD exposure (> 0.25 ppm). As we previously explained for Figure 1, we have excluded from Figure 3 the two individuals with aberrantly high badge readings. The lab worker with the contaminated badge had an M1 level of only 954 ng/mg of creatinine, and the pipefitter who was wearing a respirator at the time of his high exposure had an M1 level of 2915 ng/mg of creatinine. If the measurements from these two workers are excluded, a fairly strong positive relationship between exposure and urine M1 concentration can be seen. The sample correlation coefficient is $r = 0.68$ [95% confidence interval (CI), 0.48–0.82; $p < 0.00001$].

The relationship of *HPRT*Vf with measurable BD exposure is displayed in Figure 4. Again, the two individuals with very high badge BD levels are not included. The laboratory worker's Vf was 1.14×10^{-6} , and the pipefitter's Vf was 4.54×10^{-6} . These are reasonable frequencies because *HPRT*Vf should reflect exposure over an extended period of time, and the unusual 1-day BD exposure values for these two subjects are probably not typical for these individuals. If the two high badge values are excluded from the calculation, the sample correlation coefficient is $r = 0.51$ (95% CI, 0.26–0.70; $p = 0.0002$).

The relationship between M1 and *HPRT*Vf is shown in Figure 5. Each data point represents an individual who is identified with respect to exposure group and smoking status. Visually, there appears to be a good deal of scatter in the data. However, the individuals in the low-exposure group are clustered in the lower left quadrant of the graph, and many in the exposed group are in the upper right

quadrant. Overall, the sample correlation coefficient between the two parameters is $r = 0.42$ (95% CI, 0.14–0.64; $p = 0.00045$). The data set was also analyzed for relationships between the exposure and effect end points and other parameters including alcohol use, age, and longevity of work at this facility. No correlations were seen between alcohol use and *HPRT*Vf in any exposure group, but most users of alcohol claimed to be light or moderate drinkers. Both age and longevity were correlated with *HPRT*Vf in only one subgroup, the five smokers in the high-exposure group. The sample correlation coefficient was $r = 0.93$ (95% CI, 0.2–0.99; nonsignificant) for age and 0.98 (95% CI, 0.73–0.99; $p = 0.0012$) for longevity in the workplace. There was no correlation of *HPRT*Vf with

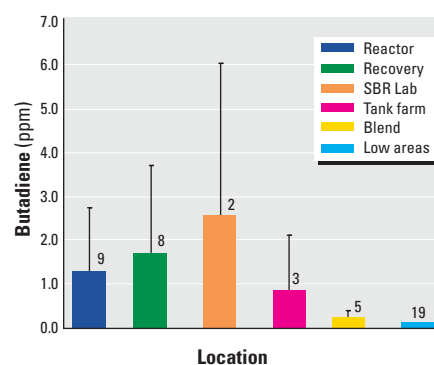


Figure 1. Average exposures based on 8-hr shift time-weighted average measurements. Error bars indicate SD. The number of samples in each work area are shown above the bars. The low-exposure areas consisted of the coagulation, baling, shipping, control room, and utility areas. All low-exposure areas samples were below the detection limit of 0.25 ppm, so a value of 0.125 ppm was assigned. The two extreme values described in the text are omitted. They were values from a worker in the styrene-butadiene rubber (SBR) laboratory and a maintenance mechanic working in several locations.

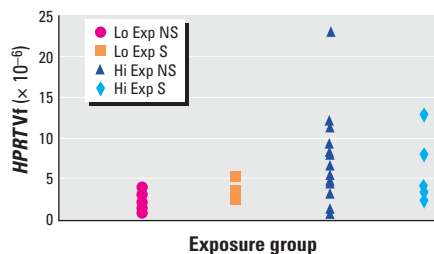


Figure 2. The distribution of frequencies of *HPRT* mutant lymphocytes is shown, stratified by exposure group and smoking status. Abbreviations: NS, nonsmoker; S, smoker. The high-exposure group consisted of workers in the tank farm, reactor, recovery, and styrene-butadiene rubber laboratory areas; the low-exposure groups consisted of workers in the blending, coagulation, shipping, control room, and utility areas. The mean *HPRT*Vfs for low exposure, nonsmokers and high exposure, nonsmokers are significantly different ($p < 0.0005$).

Table 2. Group means for BD exposure, urine M1 metabolite, and *HPRT*Vf by exposure group, with *HPRT*Vf subdivided by smoking status

Exposure group	BD (n)	Butadiene exposure (ppm) (\pm SE) ^a	Urine M1 (n)	M1 (ng/mg creatinine) (\pm SE)	Smoking status	<i>HPRT</i> Vf (n)	<i>HPRT</i> Vf $\times 10^{-6}$ (\pm SE)
High	22 ^b	1.48 (0.37)*	24	2,046 (348)**	Nonsmoker	19	6.8 (1.2)**
					Smoker	5	6.1 (2.0)
Low	24 ^c	0.15 (0.02)	23 ^d	585 (98)	Nonsmoker	20	1.8 (0.2)
					Smoker	5	3.3 (0.5)

^aValues shown are 8-hr time-weighted average exposure; results returned as below the detection limit were set to 0.125 ppm (half the detection limit) for calculation. ^bExcludes two unusually high values of 20.8 and 23.0 ppm that are discussed in the text; if those values are included, the average BD exposure is 3.18 ± 1.23 ppm. ^cBD exposure data were not obtained from one subject (M1 595 ng; Vf 2.82×10^{-6}). ^dTwo subjects failed to provide urine samples. *Significantly different from low-exposure group, $p < 0.002$. **Significantly different from low-exposure group, $p < 0.0005$.

age or longevity in the nonsmokers or in the overall group. This is important because the average age and longevity were somewhat greater in the high-exposure group than in the low-exposure group (Table 1).

Discussion

The use of biological markers offers a method for assessing human exposure to potentially hazardous agents that facilitates the evaluation of exposures at the time of their occurrence. The biological events that are used as biomarkers reflect different stages in the continuum of events that occur between human exposure to a chemical and a resulting adverse health outcome. Because these biomarkers typically respond to exposure in a much larger proportion of an exposed population than the fraction that eventually becomes ill, biomarker studies can be conducted in much smaller populations than would be required for epidemiologic studies based on morbidity or mortality as an outcome. The Committee on Biological Markers of the National Research Council has classified biological markers into categories of exposure, effect, and susceptibility (30).

In this study, we compared biological markers of both exposure (urine M1) and effect (*HPRT* mutation) to exposures measured with passive badge dosimeters. Participating workers were categorized into areas of high and low exposure to BD based on historical data. The exposure sampling showed that average exposures in the designated high-exposure areas were 1.48 ppm of BD, whereas the exposures in the designated low-exposure areas were almost all below the quantitation limit for the assay method used (about 0.25 ppm). The BD levels in the high-exposure areas can be compared to the current OSHA permissible exposure limit of 1 ppm. At the time the study was conducted, however, the standard was 1,000 ppm.

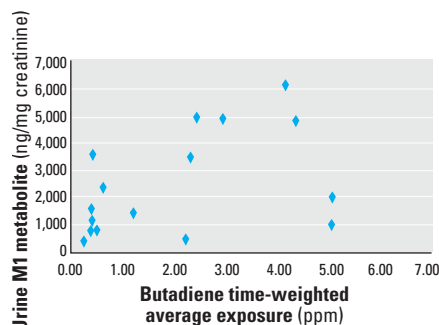


Figure 3. Relationship between the 8-hr time-weighted-average exposure to BD and the urine M1 metabolite (ng/mg creatinine). Only samples with BD levels above the detection limit (0.25 ppm) are shown, and two extremely high exposures are excluded as described in the text. The sample correlation coefficient for the data shown is $r = 0.68$ ($p < 0.00001$).

At the average exposure level of 1.48 ppm (excluding two extreme values), the two biomarkers that we used detected statistically significant increases in the concentration of the M1 metabolite of butadiene in urine, and the *HPRT* variant frequency in lymphocytes. The average M1 concentration was about 3-fold greater in the high-exposure group than in the low-exposure group. The magnitude of the difference in the *HPRT* Vf between the two groups was also about 3-fold. The BD metabolite biomarker in urine documented that the workers in the high-exposure areas received a greater internal exposure to BD than did workers in low-exposure areas. The increased frequency of *HPRT* mutant lymphocytes indicated that BD exposure was associated with an increased level of genetic damage, resulting in the fixation of mutations. Together these results suggest that occupational exposure to BD at about 1–3 ppm can be detected with biomarkers and that this exposure is associated with the induction of mutations in lymphocytes.

We found that the three main end points, measuring external and internal BD exposure and mutation as an effect of exposure, were correlated with each other. The correlations between the end points were positive, with coefficients in the range of 0.4–0.6. It is not surprising that the correlations were not stronger. The measurement of BD levels in the air using personal organic vapor monitors provides a measure of the worker's potential exposure to BD, but several factors may influence the relationship between the exposure measured by the badge dosimeter and by the output of the M1 metabolite in the urine. The badge is near the face but may not accurately reflect the breathing zone concentration under all circumstances. As we observed in one instance, a spill of BD-saturated liquid resulted in a very high badge reading, which was not reflected in the concentration of urinary metabolite.

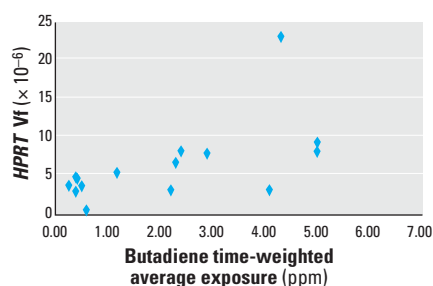


Figure 4. Relationship between the 8-hr time-weighted-average BD exposure and the *HPRT* Vf. Only samples with BD levels above the detection limit (0.25 ppm) are shown, and two extremely high exposures are excluded as described in the text. The sample correlation coefficient for the data shown is $r = 0.51$ ($p = 0.002$).

The activities of the worker may influence the absorption of BD from the air. High levels of exertion would increase respiratory rate and volume and increase the amount of BD absorbed in a unit of time. The timing of exposure would also influence the relationship between the exposure measured in the air and the concentration of the urinary metabolite. The time of peak excretion of metabolites after exposure to BD has not been determined in humans. However, BD from an exposure that occurred during the early part of the work shift would probably be excreted, in part, before the end of the shift when the urine sample was obtained. A better correspondence between exposure and the urine metabolite would probably be obtained if all the urine voided over a 24-hr period, beginning at the start of the work period, were analyzed.

Finally, it should be noted that all workers excreted at least a minimal amount of the M1 metabolite. In our first study of BD exposure, subjects who did not work at all with BD excreted a similar concentration of M1 (20). This raises the possibility that some M1 is derived from either exogenous or endogenous sources other than BD (23).

The relationship among the M1 concentration, air concentration of BD, and the *HPRT* Vf also shows substantial variability. This is not unexpected because of the timing of the manifestation of these measurements. The badge dosimeter measures the exposure during a specific exposure period, which was one 8-hr shift in this study. The urinary metabolite probably measures exposure primarily on the day of specimen collection, and not more than 1 or 2 days before collection. The time period over which *HPRT* mutant lymphocytes become manifest after an exposure, under these conditions, is not well characterized. It is known from prospective studies of patients receiving mutagenic chemical or radiation therapies that the mutant phenotype is not manifested until

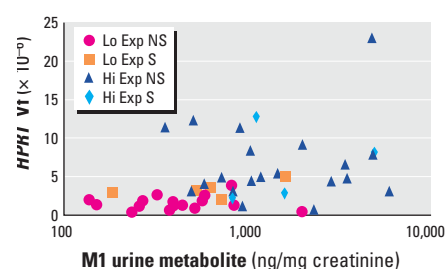


Figure 5. Relationship between the M1 urine metabolite concentration (ng/mg creatinine) and the *HPRT* Vf. The exposure group, as described in the text and in Figure 2, and smoking status are indicated. The overall sample correlation coefficient is $r = 0.42$ ($p = 0.0045$).

1–2 weeks after exposure. Also, after an acute exposure to mutagens, the frequency of mutants declines to levels far below the peak level within a few months (26,28). However, the effects of chronic exposure at relatively low doses may be different because low exposures do not produce major cytotoxic effects on lymphocyte populations. A study of former smokers showed that the *HPRT* Vf was similar in never-smokers and former smokers, but almost all of the former smokers had abstained from smoking for 5 years or more (27). Memory T-lymphocytes may persist in circulation for most of the life span of an individual. It is thus possible that some mutant lymphocytes attributable to occupational exposure could accumulate over an extended, but uncharacterized, period of time. The exposure that was measured on a single day by badge dosimetry may not reflect the cumulative exposure for the worker over a longer period of time. In subsequent studies multiple measurements of BD exposure were made over a longer period of time (31). The fact that a positive correlation was observed between exposure, measured both externally and internally, and *HPRT* Vf indicates that occupational exposure to BD did produce mutagenic effects.

Several factors have the potential to bias the results of the study, particularly with respect to the mutation biomarker. The two best characterized influences on the frequencies of *HPRT* mutants are smoking (24,27,29) and age (29,32). In earlier studies, smoking increased the *HPRT* Vf about 3-fold over the background rate. In this study we observed an increase of about 2-fold in low-exposure smokers as compared to low-exposure non-smokers. High-exposure smokers had about the same *HPRT* Vf as high-exposure non-smokers. However, there was considerable variation among the workers in their tobacco usage. The number of cigarettes usually smoked per day ranged from 6 to 40, and cotinine values ranged from 132 ng to 625 ng/mL of plasma. Mean tobacco use was about equal between the low- and high-exposure workers, but BD exposure in the five high-exposure smokers was only 0.64 ppm compared to 1.48 ppm for all of the high-exposure workers. Although smoking probably contributed to the overall frequency of mutants in the high-exposure smokers, the effect may have been offset by the lower than average BD exposure in this subgroup.

With age, the frequency of *HPRT* mutant lymphocytes increases slowly. In the autoradiographic assay the rate of increase is about 0.04×10^{-6} per year (29). In the cloning assay the effect of age is a little stronger (32). We evaluated the effect of age in the workers in this study, but found no overall effect of age on *HPRT* Vf. However, in one subgroup, the

high-exposure smokers, strong correlations of *HPRT* Vf with both age ($r = 0.93$) and longevity in the workplace ($r = 0.98$) were observed. This subgroup contained only five individuals, and the association was not observed in other subgroups, so the significance of this observation is not clear. If some *HPRT* mutant lymphocytes persist for a long period of time, they might accumulate with years of exposure to both butadiene and the mutagens in cigarette smoke.

Lymphocytes from some of the non-smoking, high-exposed workers were also assayed for *HPRT* mutant frequencies using the cloning assay (33,34). Clones of mutant lymphocytes were expanded and analyzed to determine what types of mutations they contained. They were compared to clones of mutant lymphocytes in control subjects who did not work in the petrochemical industry. Results of this study have been published separately (35). The mutant frequency (Mf) in the cloning assay in 10 high-exposure workers was significantly higher ($p < 0.05$) than the Mf in 11 controls (Mf \pm SD = $17.63 \pm 5.05 \times 10^{-6}$ vs. $8.47 \pm 2.88 \times 10^{-6}$, respectively). Autoradiographic *HPRT* assays of lymphocytes from the same samples also showed a significant increase in BD-exposed workers (Vf $6.86 \pm 3.25 \times 10^{-6}$) compared to unexposed controls ($2.36 \pm 1.04 \times 10^{-6}$; $p < 0.05$). Results from the two types of *HPRT* assays, performed on split samples, were significantly correlated ($r = 0.70$, $p < 0.001$) (36). In BD-exposed workers, a significantly higher proportion of mutant clones contained deletions of one or more exons ($p < 0.05$). A 2-fold, but nonsignificant, increase in the proportion of base substitution mutations at A-T base pairs was observed, and a higher proportion of frameshift mutations was seen in the exposed group ($p < 0.05$) (35). These observations are consistent with the types of mutations induced by BD and DEB in cell culture (16,37) and in animals (11,12,17,38). The observation of this pattern of mutation supports the idea that at least some of the mutations observed in these workers are attributable to the mutagenic activity of butadiene.

An obvious question raised by the observation of a mutagenic effect associated with exposure to a known carcinogen is whether workers exposed to low concentrations of BD have an elevated cancer risk. Concerns about cancer risk must be tempered for several reasons. First, a direct link between increased frequencies of *HPRT* mutant lymphocytes and elevated cancer risk has not been established. The only genetic biomarker for which such a link exists at this time is structural chromosome aberrations. Prospective studies have been conducted that demonstrate that individuals who developed

cancer had previously demonstrated higher rates of chromosome damage (39,40). Despite the lack of similar prospective studies of somatic cell mutations in humans, the role of mutation in carcinogenesis is well known. Documentation of an increased frequency of mutant lymphocytes that can be attributed to exposure is cause for concern. These effects, however, must be considered on a population rather than on an individual basis. The biomarker results for an individual cannot be used to predict his or her future disease risks; however, the average results for the population may be indicative of risks to the population as a whole. Also, the results of a biomonitoring study do not reflect the cumulative effect of long-term exposure, and recent exposures might not be typical of long-term experience for an individual. Other exposures, such as smoking, may contribute to the response of the effect biomarker. In addition, the genetic characteristics of individuals will quite likely influence their response to butadiene exposure. Such characteristics were not analyzed in this population; however, in continuing studies of SBR workers, we are evaluating genetic polymorphisms in genes encoding several biotransformation enzymes (41).

The results of this study are consistent with our earlier studies of workers in a butadiene monomer production facility, in which we observed an increase in *HPRT* Vf in workers in production areas. This increase in the frequency of mutant lymphocytes was correlated with an increase in the M1 urine metabolite (20,22). In continuing studies we are enlarging the study population and making a more detailed assessment of exposure using a more sensitive method for the measurement of external exposure. Our results at this point consistently indicate that exposure to BD at about 1–3 ppm is associated with an increase in *HPRT* mutant frequency.

Although our genetic biomonitoring studies of BD-exposed workers in Texas have been consistently positive to date, other studies have not detected mutagenic effects of occupational exposure to BD. A population of BD-exposed workers in China was recently evaluated (42). Reported exposures were somewhat higher than we observed in our study, with some very high episodic exposures associated with activities such as pump repair and process sample collection. Most of the male subjects in the study smoked, but none of the women used tobacco. Hayes et al. (42) found no difference between the butadiene-exposed group and the control group in the frequency of *HPRT* mutants using the cloning assay. The Mfs in both the exposed and control groups averaged $15\text{--}20 \times 10^{-6}$, which is much higher than the Mfs for the unexposed control group in our cloning assay

study (35). Tate et al. (43) studied workers in a butadiene monomer production unit near Prague in the Czech Republic. The median BD exposure, measured in 1994 for 19 workers, was 0.24 ppm, which is less than the median for the high-exposure workers in the present study. Using the cloning *HPRT* assay, no significant difference was observed between the Mfs of exposed workers and control subjects. In this case the *HPRT* Mfs for control subjects were in the same range as our cloning study, but the Mfs for BD-exposed subjects were relatively low as well. It is not clear why we have repeatedly observed an association between BD exposure and frequencies of *HPRT* mutant lymphocytes in our studies while other investigators have not. In the case of the samples from the current study, significant increases in mutant frequency were measured using both the autoradiographic and cloning assays (35). In this study, correlations among BD exposure, internal exposure, and genotoxicity were clearly observed in a facility where exposures were near the current OSHA permissible exposure limit. Because of the role of mutation in carcinogenesis, continued study of the potential carcinogenic effects of occupational exposure to butadiene, and consideration of the classification of this important industrial chemical as a carcinogen are warranted.

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